THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 53, No. 4

Printed in U.S.A.

DISTRIBUTION OF ^{3}H-BETAMETHASONE-17-VALERATE AFTER TOPICAL APPLICATION IN THE DOMESTIC PIG*

JEAN-PIERRE DESGROSEILLIERS, M.D.,† GEORGE M. LING, PH.D.,
GUY BRISSON, M.Sc. AND JANET SRETER, M.S.

ABSTRACT

The percutaneous absorption of ^{3}H-betamethasone-17-valerate was studied in young domestic pigs. Our findings suggest that this animal may serve as a suitable model for studying the absorption of steroids from the skin. A single application of radioactive cream was applied to the skin and kept there for three days under occlusion. Urine proved to be the main excretory route of this topical steroid. Excretion in feces was ten times less. After three days occlusion, an appreciable amount of radioactivity was recovered from all tissues; highest concentrations were present in the liver. Plasma samples revealed significant levels of radioactivity as early as two hours after application and reached their highest concentrations at the end of 72 hours. After a single application, maximal levels of radioactivity were also observed to persist in plasma for a period of nine days in the presence of constant occlusion. These findings are discussed with reference to the clinical use of topically applied steroids.

The beneficial therapeutic effects of steroids have been well demonstrated. However relatively little is known about plasma and tissue levels of these compounds when they are applied to the skin.

The study of the distribution of steroids applied topically may therefore be able to provide additional information about their absorption and undesirable systemic effects.

Clinically, side effects such as sodium retention and weight gain have been reported by Fitzpatrick *et al.* (1) and by Livingood *et al.* (2) after the percutaneous absorption of fludrocortisone. Scoggins and Kliman (3) observed pituitary-adrenal suppression and decreases in endogenous cortisol levels following the topical application of corticosteroids.

In addition, information about the distribution of steroids into various body compartments has been obtained with the use of labeled compounds which permit the measurement of radioactivity in body fluids. Thus Malkinson *et al.* (4) showed that radioactivity was present in the urine after hydrocortisone-4-^{14}C was applied to normal human skin. Similar observations have been made for ^{14}C-hydrocortisone by Feldmann and Maibach (5), for ^{14}C-triamcinolone acetonide by Malkinson and Kirschenbaum (6) and for ^{3}H-betamethasone-17-valerate by Butler (7).

However, reports on the plasma, fecal and tissue distribution of topically administered steroids are lacking, although Florini *et al.* (8) studied plasma half-life and excretion of ^{3}H-triamcinolone in dogs and rats, and tissue distribution in the rat, after the intravenous administration of this compound.

In this study, we report the concentration and distribution of betamethasone-17-valerate in plasma, urine, feces and tissues when this tritium labeled steroid is applied to the skin of the domestic pig.

MATERIALS AND METHODS

Domestic piglets of either sex varying in weight from 20 to 48 pounds were used in this study. Radioactive steroid cream was made with tritium labeled betamethasone-17-valerate, with a specific activity of 3.3 mc/mg (New England Nuclear Corporation of Boston, Mass.). This product was purified further using thin layer chromatography in a solvent system of ethyl acetate/cyclohexane, 2:1. Approximately 60 micrograms of purified tritiated betamethasone-17-valerate of known disintegrations per minute (DPM) was incorporated

This study was supported by the Medical Research Council of Canada, the Ontario Mental Health Foundation and Schering Corporation Limited.

We thank Dr. D. S. Layne and Dr. M. Mac Conaill for their help and advice in this study.

Received April 28, 1969; accepted for publication May 12th, 1969.

* From the Department of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada.

† Fellow, Medical Research Council of Canada. Present address: Department of Dermatology, Montreal General Hospital, Montreal, Quebec, Canada.

into 9.0 gm of a cream base identical in composition to that used by Schering Corporation in the preparation of Celestoderm Cream®.

Under Halothane® anesthesia, the skin of the back of each pig was shaved and irritated with sandpaper. The radioactive betamethasone cream was then applied to a six by six inch area which was covered immediately with Saran Wrap® (Dow Chemical Corp.) and a protective dressing. Eight pigs treated in this manner were kept in metabolic cages for the duration of this study. Saran Wrap® dressings were left on the backs of the first seven pigs for 72 hours and on an additional eighth pig for 9 days.

Blood samples of 1 ml were obtained from the ear veins and collected in heparinized tubes at 2, 4, 24, 30, 48, 54 and 72 hours. The plasma was removed, frozen immediately and stored at −10° C. Twenty-four hour urine and feces samples were also frozen and stored for future analyses.

At the end of each 72 hour treatment period, pigs were anesthetized and exsanguinated to minimize radioactive contamination of tissues and organs removed during autopsy. With the exception of the gastro-intestinal tract, all organs were weighed and samples of approximately 50 mg were taken for evaluation of their radioactive content. Small pieces of the Saran Wrap® and skin at the site of application were also removed for analysis. 0.4 ml of NCS solubilizer®, (a quaternary ammonium base in a 0.6 N Toluene solution) obtained from Nuclear Chicago Corporation was added to the samples of Saran Wrap® and skin, and 0.2 ml was added to all organ and tissue samples to digest them. The steroid, betamethasone-17-valerate was extracted from feces with methanol.

Modified Bray's solution[1] (10 ml) was added to all plasma, urine, feces and digested organ samples, and radioactive determinations were made with a Mark 1 Nuclear Chicago Liquid Scintillation Counter. The channels ratio method was used to correct all samples for variations in counting efficiencies; the results were expressed as disintegrations per minute (DPM).

Since in a number of cases, the percentage of the absorbed dose showed wide variations and yet only positive values were meaningful, these measurements were considered as being log normally distributed. The arithmetic means and standard errors were calculated for the logarithms of these measurements, and these statistics reconverted to the original percentage scale giving geometric means with their equivalent standard errors.

RESULTS

Figure 1 illustrates the mean levels of radioactivity (five pigs) recovered in plasma at 2, 4, 24, 30, 48, 54 and 72 hour intervals. The graph is expressed as a percentage of the absorbed DPM and shows significant values at 2 hours with a linear increase thereafter to attain the highest value at 72 hours (0.68 ± 0.13%).

Table I indicates that at the end of 72 hours, 11.0 ± 2.3% of the total DPM absorbed had been recovered in the urine; 1.4 ± 0.4% was present in the feces and the content of the organs studied accounted for 6.8 ± 2.7%. When expressed as a percentage of the total DPM applied, the plasma, urine, feces and organs radioactivity amounted to 19.9 ± 3.6%. The Saran Wrap® and the six by six inch piece of skin at the site of application yielded 12.2 ± 3.5% of the total DPM applied. Thus, a total of 32.1 ± 5.0% of the initial radioactivity was recovered. In this study, no attempts were made to determine the amount of radioactivity in the muscle mass, skeleton and other areas of the skin; it is therefore possible that the remaining 67.9% of the total dose applied may be partially localized in these structures, and in part either secreted by sweat glands or lost during expiration.

The evaluation of the tissue distribution of labeled betamethasone-17-valerate presented certain difficulties. Severe quenching occurred from the use of a potent quaternary ammonium base tissue-dissolving reagent and as a result of the yellow color of the chemically treated tissues. In the first pig, there was an apparent high concentration of activity in the pituitary and adrenals when compared to other tissues. Subsequent animals did not show this preferential concentration. The greatest activity was concentrated in the liver and the total mean recovery in the organs studied was 6.8 ± 2.7%.

In order to evaluate the reproducibility of our experimental technique, two pigs (numbers 6 and 7) of approximately the same size and weight (14.3 and 12.2 kg) were topically treated with radioactive cream containing respectively 380 × 10^6 DPM and 360 × 10^6 DPM, using the experimental conditions outlined in materials and methods. Figure 2 illustrates average levels of radioactivity obtained from the urine and feces of the two pigs used in this experiment. These levels were similar in both pigs with a maximal daily urinary excretion at 24 hours and maximal fecal activity at 48 hours. In the urine, pig 6 excreted 15.1 × 10^6 DPM during the first 24 hours while pig 7 had a value of 13.6 × 10^6 DPM during the same period. Plasma levels of radioactivity were also similar

[1] 1 gallon Dioxane, 400 gm Naphthalene, 28 gm PPO, 1.2 gm POPOP.

in both pigs and reached their maximal levels at 72 hours; at the end of this period, pigs 6 and 7 showed values of 2.9×10^6 and 2.1×10^6 DPM respectively. It is of interest that the urine output of radioactivity reached its peak level before the plasma level had attained its maximal value. One possible explanation for this observation may be related to the fact that plasma samples were collected and analysed at specific time intervals, whereas urine values represent voided urine which was collected for 24 hours and analysed at the end of that period. An indwelling urinary catheter which would permit collection of urinary samples at specific time periods should provide a more accurate reflection of a time course relationship between plasma and urinary excretion values.

Since the level of radioactivity in the plasma showed maximal values at the end of the 72 hour period, it was of interest to determine whether further increase in plasma radioactivity would be present over a longer period. To test this possibility, the Saran Wrap® and protective dressing were left on an additional pig for a period of 9 days. Plasma and urine samples were taken daily; these results are illustrated in figure 3. The highest radioactivity in plasma occurred on the second day and was 4.0×10^6 DPM. Minor fluctuations from this value were observed for the following week, and 3.2×10^6 DPM was obtained on the 9th day. Urine, however, showed marked changes daily. The peak daily excretion of 28×10^6 DPM occured on the 2nd day and decreased to a final value on the 9th day of 1.6×10^6 DPM. The total cumulative (for nine days) urinary radioactivity was 72×10^6 DPM (15% of total DPM applied).

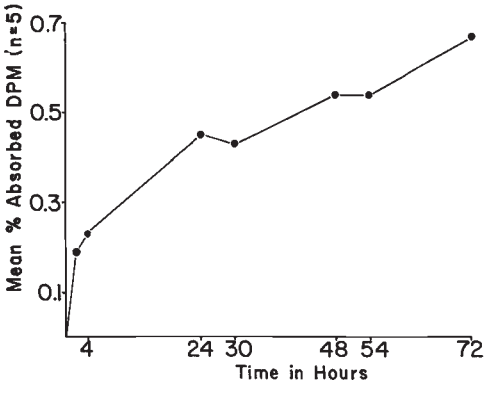

FIG. 1. Mean levels of radioactivity in plasma

TABLE I

Percutaneous absorption of ^3H betamethasone-17-valerate after 72 hrs. application with occlusion

% of	Total DPM absorbed	% of total DPM applied
Plasma	0.68 ± 0.13†	
Urine	11.0 ± 2.3	
Feces	1.4 ± 0.4	
Organs	6.8 ± 2.7	
SR + SK*		12.2 ± 3.5
P + U + F + O		19.9 ± 3.6
P + U + F + O + SR + SK		32.1 ± 5.0

* SR = Saran wrap, SK = Skin, P = Plasma, U = Urine, F = Feces, O = Organs.

† Geometric Mean ± Equivalent Standard Error.

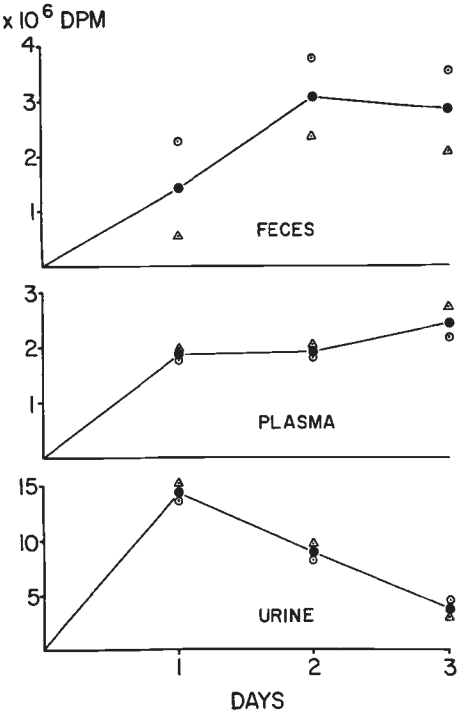

FIG. 2. Levels of radioactivity. The solid line represents the average of values obtained in two pigs. Triangle: Pig #6. Circle: Pig #7.

DISCUSSION

The results indicate clearly that betamethasone-17-valerate is absorbed from the skin of

the domestic pig, and that significant levels can be found in plasma two hours after application. Since some similarities exist between porcine and human skin it is likely that a similar rate of percutaneous absorption may also occur in man; however, histological dissimilarities have also been pointed out by Montagna (9) and further comparative studies of the absorptive characteristics of the skin of man and the pig are needed.

Over a 72 hour period plasma levels of radioactivity showed an initial rapid increase and gradually reached maximal activity at 72 hours. The initial entry of the labeled steroid into circulation may be due, in part, to rapid absorption via the follicular pathway as described by Feldmann and Maibach (5), and in part, to the experimental condition of the animal's skin which was slightly irritated to produce hyperemia, often associated with inflammatory reactions of the skin. Comparison of the histological analysis of normal skin with that of the skin treated lightly with sandpaper (5 strokes in either direction) did not reveal any appreciable differences; mild erythema was observed when the skin was irritated but no damage to the stratum corneum was visible under microscopic examination. This area was also occluded with Saran Wrap® and clear evidence has been presented by McKenzie and Stoughton (10) that such a procedure will enhance the absorption of topical steroids 100-fold.

Our observation that high plasma levels obtained at 72 hours continued to persist until the ninth day is also of interest. Vickers (11) has described a stratum corneum reservoir for topically applied steroids which is capable of releasing the betamethasone-17-valerate when permissive conditions such as occlusion are in constant operation. It is also possible that the consistently high plasma levels of radioactivity may have resulted from tissue sources. Cope (12) has described the uncertain fate of steroids located in tissues. He suggests that they are held there for a short time and are then released with or without chemical change. This view has also been discussed by Tait *et al.* (13). Florini and his co-workers (8) noted that after intravenous administration, the labeled steroid was rapidly taken up by muscle and was equal in concentration to that of blood. After two hours, the amount of steroid in blood was less

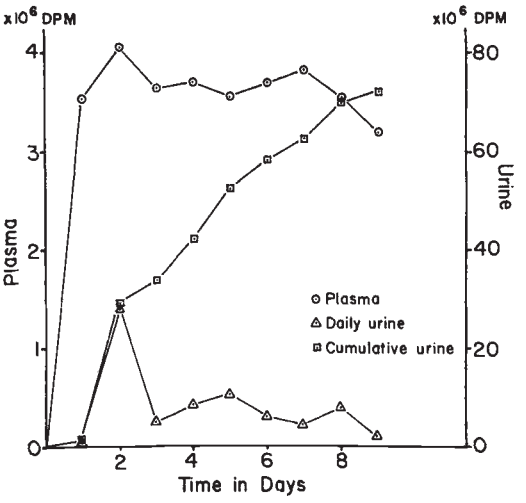

FIG. 3. Levels of radioactivity in plasma and urine during nine days of occlusion.

than that found in muscle, supporting the possibility that the labeled steroid or its metabolic products may be more firmly bound to muscle than to blood proteins.

The percentage of betamethasone-17-valerate excreted in the urine of the pig was 11.0 ± 2.3%. This value is similar to that observed in man. Studies by Butler (7) have shown that urine is the main excretory route of betamethasone-17-valerate. In her studies, a patient with pemphigus who had 20% of the body area occluded for 3 days, excreted 18.5% of the total applied dose. In our experiments, 1.4 ± 0.4% of the total absorbed dose was recovered in feces and this is in agreement with reports in man (7) where similar percentages (1.6 to 3.5%) were found in feces after intravenous or oral administration of ^{3}H-betamethasone-17-valerate.

When a single application of labeled steroid cream was kept under occlusion for three days, the plasma levels of activity progressively increased and reached maximal values at 72 hours. In these pigs, 12.2 ± 3.5% of the total dose applied still remained on the Saran Wrap® and at the skin site of application. The results obtained when a single application of ^{3}H-betamethasone-17-valerate cream was left in place, under occlusion, for nine days are also of interest. Under these conditions, the plasma radioactivity was maintained at consistently high levels throughout the duration of the experiment, and it is possible that the stratum

corneum reservoir described by Vickers (11) may influence steroid plasma levels when permissive conditions such as occlusion are in constant operation. This possibility has been raised by Kirketerp (14) who suggested utilizing this reservoir therapeutically by changing the occlusive plastic film dressing daily while applying the steroid cream every third or fourth day. Scoggins and Kliman (15) have made the interesting observation that in some concentrations the percutaneous steroid dose necessary to bring about adrenal suppression is quite close to the suppressing oral dose. This suggested to them that a given dose could be more potent when given percutaneously than when given intermittently by the oral route. The "alternate-day" regime of oral corticosteroid therapy which was proposed independently by Reichling *et al.* (16) and by Harter *et al.* (17) has succeeded in reducing the incidence and severity of clinical side effects without minimizing the desired therapeutic benefits. The possibility exists that a similar treatment schedule using topical steroids could yield results of clinical importance.

REFERENCES

1. Fitzpatrick, T. B., Griswold, H. C. and Hicks, J. H.: Sodium retention and edema from percutaneous absorption of fludrocortisone acetate. J.A.M.A., *158:* 1149, 1955.
2. Livingood, C. S., Hildebrand, J. F., Key, J. S. and Smith, R. W.: Studies on the percutaneous absorption of fludrocortisone. Arch. Derm., *72:* 313, 1955.
3. Scoggins, R. B. and Kliman, B.: Percutaneous absorption of corticosteroids: Systemic effects. New Eng. J. Med., *273:* 831, 1965.
4. Malkinson, F. D. and Ferguson, E. H.: Percutaneous absorption of hydrocortisone-4-C^{14} in two human subjects. J. Invest. Derm., *25:* 281, 1955.
5. Feldmann, R. J. and Maibach, H. I.: Penetration of ^{14}C-hydrocortisone through normal skin. Arch. Derm., *91:* 661, 1965.
6. Malkinson, F. D. and Kirschenbaum, M. B.: Percutaneous absorption of ^{14}C-labeled triamcinolone acetonide. Arch. Derm., *88:* 427, 1963.
7. Butler, J.: Percutaneous absorption of tritium labeled betamethasone-17-valerate. Brit. J. Derm., *78:* 665, 1966.
8. Florini, J. R., Peets, E. A. and Buyske, D. A.: Plasma half-life tissue distribution and excretion of triamcinolone-H^3. J. Pharm. Exp. Ther., *131:* 287, 1961.
9. Montagna, W. and Yun, J. S.: The skin of the domestic pig. J. Invest. Derm., *43:* 11, 1964.
10. McKenzie, A. W. and Stoughton, R. B.: Method for comparing percutaneous absorption of steroids. Arch. Derm., *86:* 608, 1962.
11. Vickers, C. F. H.: Existence of reservoir in the stratum corneum. Arch. Derm., *88:* 20, 1963.
12. Cope, C. L.: The adrenal cortex, *Chemistry and Pharmacology of the Adrenocorticol Hormones.* Proceedings Symposium Royal Society of Medicine, London, Oct. 14–15, 1960. Published by J. B. Lippincott.
13. Tait, J. F., Tait, S. A. S., Little, B. and Laumas, K.: The metabolism of aldosterone in man, *The Human Adrenal Cortex.* Proceedings of a conference, University of Glasgow, July 11–14, 1960. Published by E. & S. Livingstone Ltd.
14. Kirketerp, M.: Systemic effects of local treatment with flucinolone acetonide applied under plastic film. Acta Dermatovener., *44:* 54, 1964.
15. Scoggins, R. B. and Kliman, B.: Relative potency of percutaneously absorbed corticosteroids in the suppression pituitary-adrenal function. J. Invest. Derm., *45:* 347, 1965.
16. Reichling, G. H. and Kligman, A. M.: Alternate day corticosteroid therapy. Arch. Derm., *83:* 134, 1961.
17. Harter, J. G., Reddy, J. G. and Thorn, G. W.: Studies on an intermittent corticosteroid dosage regimen. New Eng. J. Med., *269:* 591, 1963.